Project Description

------ Hepatitis C virus detection test

Brainstorming and Selection

Inspired by Olivia Hallisey's invention in 2015--Detection of Ebola via a Silk-Derived Lateral-Flow System and Mr. Keith Pardee's article about Paper-Based Synthetic Gene Networks, initially, we had the idea of designing a test paper to contribute to virus detection via the method of synthetic biology. We've made research on several viruses, including Ebola, Zika and HIV, however, when later confronted with statistics revealing the high mortality rate of HCV and the great magnitude of its spreading, China included, we shifted our attention to the relatively neglected virus. Later, Mr. Keith Pardee and his lab published an article about the detection of Zika virus which was an extension of their previous program *(Keith Pardee, 2016)*. The article has motivated us to apply Nucleic Acid Sequence Based Amplification (NASBA) to our project.

Motivation

·Hepatitis C is an infectious disease caused by the hepatitis C virus (HCV) that primarily affects the liver. *(Ryan KJ, Ray CG, eds., 2004)*The Hepatitis C virus is a blood borne virus and the most common modes of infection through unsafe injection practices.

·Hepatitis C is found worldwide. The most affected regions are Africa and Central and East Asia. Depending on the country, hepatitis C infection

can be concentrated in certain populations (for example, among drug injectors); and/or in general populations. According to WHO in 2015, an estimated 130~150 million people worldwide have chronic hepatitis C infection.*(WHO, 2015.)*While in China, 0.7%~3.1% of the healthy individuals, about 38 million people, are infected and the number continues to rise in the recent decades.*(Tongjing Xing, Hingtao Xu. 2010)*

·Since the immune system of human body is not able to clear the virus spontaneously, around 50%~80% of the infectors will have chronic hepatitis, and 20%~30% of them will develop liver cirrhosis, 1%~4% of which might turn into liver cancer. (In particular, of the five types of Hepatitis, types B and C lead to chronic disease in hundreds of millions of people and, together, are the most common cause of liver cirrhosis and cancer.) Approximately 500 000 people die each year from hepatitis C-related liver diseases.

·There are 6 strains (or genotypes) of the HCV and their distribution varies by region. In Asia, the most common types of the infection are HCV II and HCV III.*(WHO, 2015.)*

• There is currently no vaccine for hepatitis C though research in this area is ongoing. Due to the fact that acute HCV infection is usually asymptomatic, few people are diagnosed during the acute phase. In those people who go on to develop chronic HCV infection, the infection is also often undiagnosed because the infection remains asymptomatic

until decades after infection when symptoms develop secondary to serious liver damage. Thus the need for effective early-stage diagnosis methods is urgent.

• Current diagnosis methods can be categorized into Serologic Antibody Assays (including EIA, CIA, RIBA)*（Hepatitis Online,2015）*, and Molecular HCV RNA Tests（including RT-PCR, bDNA Assay. However, due to complex testing procedures and expensive equipments required, the promotion of these methods are limited.

Witnessing the current situation, we aim to create a new type of low-cost and high-sensitivity testing method combining qualitative and quantitative assay functions to be applied in military and humanitarian aid field operations, and developing countries.

The Design

Under the inspiration of Paper-Based Synthetic Gene Networks *(Keith Pardee, 2015)* we decide to create cell-free systems on paper with the fundamental transcription and translation properties of a cell but that are sterile and abiotic. Importantly, these freeze dried materials are stable over time, with transcription and translation activity remaining after a year of room temperature storage. Stable at room temperature, these embedded materials are readily stored, distributed, and can be

activated by simply adding water. Here are the two kinds of test paper we determine to engineer.

A. Qualitative Paper

Test paper A is divided into two parts. Plasmid Ⅱ and Plasmid Ⅲare input respectively to detect HCV Ⅱ and HCVⅢin Gingival crevicular fluid*(World Chin J Digestol, 2005)*will be amplified through Nucleic Acid Sequence Based Amplification(NASBA)*(Cordray and Richards-Kortum, 2012)*and will be added as substrate. Downstream lacZ reporter gene will be expressed when Trigger RNA pairs themselves with our special Toehold Switch, which turns the test paper from yellow to purple.

Toehold switch is first designed by Alexander A. Green, Pamela A. Silver, James J. Collins and Peng Yin. Like natural riboregulators, engineered riboregulators of translation have invariably used base pairing to the ribosome binding site (RBS) to prevent ribosome binding, thereby preventing translation.*(Alexander A. Green，2014)*Conventional riboregulators have historically repressed translation by sequestering the ribosomal binding site (RBS) of the transducer RNA within a hairpin. This hairpin is unwound upon binding of a cognate trigger RNA, exposing the RBS and enabling translation of the downstream protein.[Figure 1]

In order to find out the best sections of RNA to detect HCV, we chose 4 parts from each HCV RNA sequences to be our triggers, and designed 8

different Toehold Switches. Each has a domain that can pair with a specific Trigger.[Figure 2]

B. Quantitative paper

Paper B allows medical personnel to do quantitative assay. We replace lacZ reporter gene with luciferase reporter gene which can determine the relatively accurate concentration of trigger RNA. **[Figure 2]**

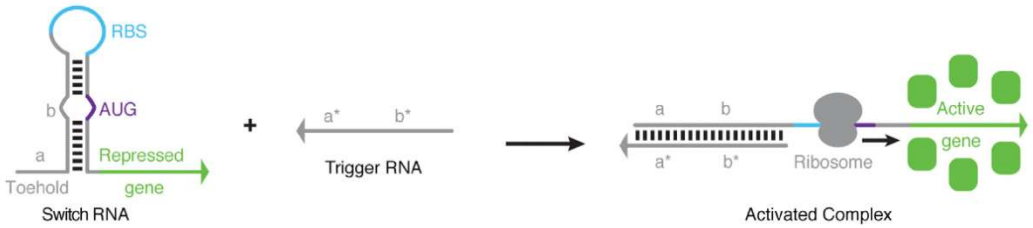

Figure 1.**Toehold Switch activated by Trigger RNA:**

Variable sequences are shown in gray, whereas conserved or constrained sequences are represented by different colors.

A. B.

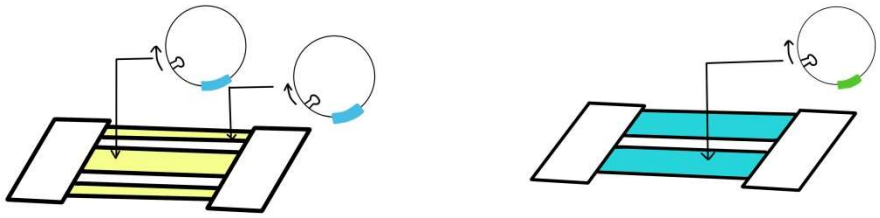

Figure 2.Test paper designs:

A. Paper A has two parts with 2 kinds of plasmid in order to detect HCV Ⅱ and Ⅲ

B. Paper B has plasmids with luciferase reporter gene

Future Work

Currently, while experiments are in progress, we are working on the construction of a quantitative paper with improved sensibility by using plasmids with dual-luciferase reporters. We hope to put the test paper into production so that more regions in the world can be covered with the advanced detection method to fight against HCV. Moreover, in the future, we hope that the synthetic biological detection method can be applied to the diagnosis of other viral diseases by changing the sequence of the toehold switches.

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